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Production and characterization of ora-pro-nóbis and agar-agar based edible leathers (Snack-films): a new plant-based food option

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ABSTRACT

The present study aimed to produce and characterize edible leathers based on ora-pro-nóbis leaf puree (OLP), with concentrations ranging from 30 to 60 g/100 g of ELFM (edible leather forming mass) and agar-agar (3 g/100 g ELFM), using the tape casting technique. The edible leathers were dried in an oven and evaluated for their physicochemical parameters (Aw, moisture, pH, protein content, and hygroscopicity), optical properties (color and browning index), structural characteristics (thickness and SEM), mechanical properties, and spectroscopic profile (NMR). Regardless of the OLP concentration used, it was possible to form leathers from ora-pro-nóbis leaves and agar-agar. Color characteristics were not significantly affected in terms of lightness (L) and chroma a. However, significant differences were observed for chroma b* and C*. The physicochemical stability of the leathers was confirmed by their low water activity (<0.8) and moisture content (<7.8 %). Increasing OLP levels resulted in higher protein content (6.8–8.9 g/100 g w.b.) and thickness (0.3–0.5 mm), which positively impacted puncture resistance and indicated a more cohesive structure. SEM analysis revealed heterogeneous surfaces and internal structures, with discontinuity zones attributed to compounds in the OLP. NMR results confirmed the presence of hemicellulose, lignin, cellulose, and amino acids, reinforcing the nutritional value of the leathers. Additionally, total phenolic content (680–830 mg GAE/g edible leathers) and ABTS•+ (1.272–1.499 μmol Trolox eq./100 g edible leathers) increased with higher OLP content, demonstrating the product's functional potential. The incorporation of ora-pro-nóbis puree into edible leather formulations contributed to improvements in the nutritional, functional, and structural aspects of the final product. Thus, the developed edible leathers are a promising alternative for using non-conventional vegetables, contributing to innovation in functional and sustainable food products.

1. Introduction

In recent years, due to the numerous health problems associated with high-calorie, low-nutritional-value diets, alternatives have been explored, emphasizing the search for greater practicality and reducing the time needed to prepare meals. In this context, the production of snacks and/or leathers (edible films), which are regarded as quick meals, has seen significant growth and often plays a significant role in providing nourishment between main meals [1]. However, excessive consumption of this type of food may have health risks, as it is typically associated with a high body mass index (BMI), eating in the absence of hunger, and dining outside the home, all of which can significantly

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contribute to excessive calorie intake [2].

Snacks are usually associated with foods that are high in energy but low in nutrients, often containing large amounts of sodium, sugar, or saturated fat (Niro et al., 2022). However, according to Chobot et al. (2024) [1], a worldwide trend in vegetable consumption is emerging, growing a market for health-conscious food products and plant-based snacks. With an estimated annual growth rate of 6.6 % from 2022 to 2030, the healthy snacks market is growing rapidly, reaching a value of US$152.3 billion (Statista, 2025).

The growing demand for healthier products is driven by increasing awareness of well-being issues. According to the literature, epidemiological studies suggest that high consumption of fruits and vegetables is associated with cancer risks and benefits for diabetes, obesity, and cardiovascular diseases (Jiménez-Cruz et al., 2002; Marangoni et al., 2019). Fruit-based snacks or leathers have emerged (Niro et al., 2022; Rodrigues et al., 2023), mainly as a substitute for fried or high-calorie products, attending to the consumer demand for practical options aligned with a healthier lifestyle.

Unlike traditional snacks, leathers (edible films) are usually made with minimal ingredients and involve processes such as drying to reduce their moisture content (Bustos et al., 2023). Leathers are advantageous because they can be easily incorporated into daily meals, often between main meals, and their consumption has expanded to include all age groups, from children to adults (Azeredo et al., 2022). They may also provide benefits such as high antioxidant capacity and antimicrobial properties, among others.

According to Bedir and Karaoglu. (2022) [3], the textural properties of edible leathers are essential for determining the quality of the final product intended for consumption. Several studies in the literature have investigated the addition of various ingredients to inprove these characteristics, including starch, wheat flour, wheat bran, and wholemeal flours [3], as well as wheat, corn, and potato flours [4]. Additionally, different hydrocolloids have been used, including Arabic gum, xanthan gum, Persian gum, gum tragacanth, gelatin, and carboxymethylcellulose [5].

In addition to the increasing population growth, the search for sustainable solutions regarding food security is directly related to FAO's Sustainable Development Goals [6]. The valorization of UFPs and the development of new products from these plants represent promising strategies to achieve goals such as the eradication of hunger (SDG 2), the promotion of health and well-being (SDG 3), and responsible consumption and production (SDG 12). Additionally, utilizing UFP species can contribute to food diversification and reduce dependence on monocultures. Including UFP in food products can stimulate local production chains, creating more sustainable and accessible food models for future generations. Among the UFPs that can be used in leathers (edible films), the ora-pro-nóbis is notable for its nutritional and functional characteristics [7].

Although ora-pro-nóbis has great potential to improve human diets, its use remains limited due to the absence of large-scale cultivation; it is mainly consumed in regions where it holds cultural importance. However, it is widely recognized for its high nutritional value and health benefits, and incorporating it into innovative products, such as edible leathers, offers a promising way to increase its consumption, add value to functional foods, and promote the use of non-conventional food plants.

In this context, the main objective of this study was to produce edible leathers with different ora-pro-nóbis purée concentrations while maintaining a constant agar-agar concentration. This work evaluated their physicochemical, structural, functional, and cytotoxic properties.

2. Material and methods

2.1. Material

Ora-pro-nóbis leaves of the species *Pereskia aculeata Mill* (BOTU 35880, Herbarium Irina Delanova De Gemtchujnicov) were used to produce the leathers. Agar-agar (vegetable gelatine) was purchased from Mercearia e Bomboniere Towa LTDA (São Paulo, SP).

2.2. Production of ora-pro-nóbis leaf puree

The ora-pro-nóbis leaf puree (OLP) was obtained from ora-pro-nóbis leaves that were manually separated from the stems. The leaves were washed in running water, immersed in a sodium hypochlorite solution (Hidrosteril, Sagdio, São Paulo, SP, Brazil) for 15 min, and then rinsed in running water to remove the sanitizing solution. Following the sanitization process, the leaves were steamed (98 °C, 100 % steam for 1 h) in a combined oven (Prática, C-Mas Gourmet, Piracicaba, SP, Brazil) and then ground in a cutter (Sammic, CKE-5, Linda-a-Velha, Portugal) at 3000 rpm for 5 min. The single batch of ora-pro-nóbis puree (POR) was packed into plastic containers (Grespan, Zip bags, 24 x 34 cm) and frozen in a freezer (Consul, CRM54, SP).

2.3. Production of edible leathers (edible films)

For the production of edible leathers, the ora-pro-nóbis leaf puree (OLP) was initially thawed for 24 h under refrigeration (Brastemp, BVR28HBBNA, SP). The edible leathers were produced with different concentrations of ora-pro-nóbis puree (30, 40, 50, and 60 g of OLP/100 g of ELFM - edible leather forming mass), with a fixed agar-agar concentration (3 g of agar-agar/100 g of ELFM). The OLP was first heated in a water bath (Marconi, MA 159, SP) until reaching 80 °C (~20 min). In comparison, the agar-agar was separately dissolved in water at 100 °C on a heating plate (IKA, C-MAG HS 7, SP) under mechanical stirring (IKA, Digital RW20, SP) at 600 rpm for 10 min (Garcia et al., 2022). Afterward, the film solution (agar-agar + water) was cooled to approximately 80 °C, and the OLP was added with mechanical agitation (IKA, Digital RW20, SP) at 1000 rpm for 10 min. The ELFM was then spread onto Teflon plates by tape-casting using an automatic spreader (ZAA 2300, Zehntner Testing Instruments, Sissach, Switzerland), operated at a constant speed of 20 mm/s. The thickness of the edible leathers was controlled with a universal applicator (TBK Erichsen) to achieve 4000 μm. The edible leathers were dried in an air-circulating oven (Marconi, MA 35) at 50 °C for 9 h. After drying, they were stored in a desiccator containing a NaBr saturated saline solution (relative humidity 58 % at 25 °C). For samples used in scanning electron microscopy (SEM) analysis, storage was extended to 10 days in a desiccator with silica.

2.4. Leathers characterization

2.4.1. Visual assessment and color parameters

Edible leathers were visually assessed for film formation, their ability to be removed from the support, and their handling characteristics. The color parameters (L*, a*, and b*) were determined using a spectrophotometer (HunterLab, Aeros). Readings were taken at 10 random points on samples with a diameter of 7 cm. In addition, the chroma C* according to Equation (2) [8], and the browning index were determined (BI, Equation (3), [9].

$$C^* = \sqrt{(a^{*2} + b^{*2})} \tag{2}$$

$$BI = \frac{100}{0,172}\left(\frac{a^* + 0,75L^*}{5,64L^* + a^* - 3,012b^*} - 0,31\right) \tag{3}$$

2.4.2. Water, moisture, and protein activity

Water activity was measured using an Aqualab water analyzer (25 °C). Moisture and protein contents were determined according to AOAC (1990) using an oven (Fanem, 315 SE) at 105 °C and a Kjeldahl apparatus (Tecnal, TE 036/1) with a correction factor of 6.25, respectively.

2.4.3. Hygroscopicity and pH

To measure hygroscopicity, samples (2 × 2 cm^2) were placed in a desiccator containing a saturated sodium sulphate solution (81 % RH) at 25 °C. After 7 days, the hygroscopicity was calculated according to Cai and Corke [10], as shown in Eq. (4).

$$Hygroscopicity = \frac{m_f - m_i}{m_i} x\ 100 \quad (1)$$

Where: Hygroscopicity= (g moisture/100 g dry edible leather); mf = final sample mass (g); mi = initial sample mass (g)

The pH was measured using a 2 g sample dispersed in 20 mL of distilled water at 100 ± 2 °C. The dispersion was stirred at 150 rpm in a shaker incubator (Marconi, MA 420) at 25 ± 2 °C for 30 min [11]. The pH was read after 1 min of contact between the electrode and the solution.

2.4.4. Thickness and perforation

Leather thickness was measured using a Mitutoyo Absolute digital indicator (Interface IT-016U, Japan) in 10 random measurements taken on the surface of the leather (10 x 10 cm). The perforation test was conducted using a texturometer (TA Instruments, TA.XT2 Plus, New Castle, USA). Leather samples (diameter 70 mm) were fixed in a perforated circular cell (internal diameter 52.6 mm). Tests were carried out with a cylindrical probe (Cylinder Stainless, P/2 = 2 mm) at a speed of 1 mm/s (Gontard et al., 1993). The perforation shape was analyzed using Exponent software version 6.1.7 (Stable Micro Systems, England).

2.4.5. Scanning electron microscopy and nuclear magnetic resonance (NMR)

The film's surface and fracture were analyzed using a scanning electron microscope (TM-3000, Hitachi, 15 kV). Samples were fixed to the equipment support with double-sided copper conductive tape. To analyze the internal structure, the films were fractured in liquid nitrogen.

Solid-state ^{13}C nuclear magnetic resonance (NMR measurements were performed on an Avance III - Bruker spectrometer with a 9.4T magnetic field (400 MHz for 1H). The spectra were obtained using the CPMAS technique, which involves cross-polarization, magic-angle spinning, and high-power decoupling. For carbon nuclei, a 90° pulse of 4.5 μs, a contact time of 1.0 ms, 1024 scans, an acquisition time of 15.0 ms, and a recycle delay of 5.0 s were used at 25 °C.

2.4.6. Total phenolic compounds and ABTS•$^+$

For the determination of total phenolic compounds and ABTS•$^+$ (Roche, Mannhein, Germany), the edible leathers (~0.4 g) were dispersed in 10 mL of 70 % ethanol (Synth, Diadema, SP, Brazil) and placed in an ultrasonic bath (Ultra cleaner, 1400 A) for 15 min. The samples were then centrifuged at 7500 rpm (4 °C). Aliquots of the supernatant (0.5 mL) were added to 2.5 mL of diluted Folin-Ciocalteu reagent (1:10; v/v, Sigma-Aldrich, Buchs, Switzerland). After 5 min, 2.0 mL of sodium carbonate solution (7.5 %, Synth, Diadema, SP, Brazil) was added. The mixture was homogenized and left to rest in the dark for 2 h (Singleton et al., 1998). The absorbance was measured using a spectrophotometer (Bel, 1105) at 740 nm, and the results were expressed in mg gallic acid (Êxodo Científica, Sumaré, SP, Brazil) equivalents (GAE)/g edible leather.

For the ABTS•$^+$, the radical was produced by reacting a solution of ABTS (7 mM) and potassium persulfate (2.45 mM), and the mixture was left to stand for 16 h [12]. After this period, 3 mL of the ABTS radical (Abs 0.7, diluted in ethanol) was added to 30 μL of the solution, and was analyzed in a spectrophotometer (Bel, 1105) at 734 nm after 6 min. The results were expressed in μmol Trolox eq./100 g edible leathers.

2.4.7. Cytotoxicity analysis

To evaluate the effects of the leathers on cell viability, fibroblasts (3T3-E1) were cultured in culture flasks containing RPMI culture medium (Gibco, Paisley, United Kingdom) supplemented with 10 % fetal bovine serum (FCS; Gibco) and 100 UI/mL of penicillin and 100 μg/mL of streptomycin (Gibco). The cells were incubated at 37 °C in a humidified atmosphere with 5 % CO_2 until reaching 80 % confluence (ISO 10993-5, 2009). Then, the cells were seeded in 96-well plates at a density of 5000 cells per well. After cell adhesion (~4 h). Samples of the edible leathers were diluted in culture medium and incubated in a shaker at room temperature for 24 and 48 h. Then, 200 μL of this solution was added to each well and kept in contact with the leathers for 24 h. Subsequently, the culture medium was then removed, and the MTT solution (5 mg/mL) was added, with the presence of formazan crystals evaluated using a microplate reader (Epoch) at 550 nm. The results for the experimental groups were compared to those of the control group. For the negative control, cells were seeded in culture medium with a 70 % alcoholic solution.

2.5. Statistical analysis

The data were statistically evaluated using analysis of variance (ANOVA). In the event of a statistical difference, Duncan's test ($p < 0.05$) was performed using the InfoStat software (Version 2018d).

3. Results and discussion

3.1. Visual evaluation and color parameters

Visually, the edible leather presented structural integrity regardless of the concentration of ora-pro-nóbis leaf puree (OLP) added, forming a heterogeneous matrix (Table 1). It was associated with the heterogeneity of the puree and OLP concentrations. The incorporation of OLP also influenced the texture and structure, supporting the integrity of the matrix and potentially enhancing its nutritional value. Additionally, the edible leathers were easy to remove from the support.

Increasing the OLP concentration did not significantly influence ($p > 0.05$) the luminosity (black to white) and chroma a* (green to red) of the edible leathers (Table 1). However, the chroma b* of the formulation with 30 C_{OLP} differed significantly from 40 C_{OLP}, indicating a reduction in yellow tonality, as observed visually. A similar significant difference was noted for Chroma C* (Table 1), suggesting lower color saturation in the formulation with 40 C_{OLP}, possibly due to the higher concentration of chlorophyll, which can mask other pigments such as yellow. According to Cruz et al. [13], ora-pro-nóbis leaves have a chlorophyll content ranging from 0.48 to 5.33 mg/g, and both the solvent and extraction method influence the extraction process [14]. reported that the green color of plants is due to the presence of chlorophylls *a* and *b*. However, when exposed to heat, these molecules can undergo changes, such as the loss of the magnesium ion, resulting in the formation of pheophytin, which alters the color and promotes darkening of the product.

No significant differences were observed in the browning index (Table 1), indicating that the puree concentration did not influence this parameter. This may be because the added OLP underwent a cooking process, establishing reactions that could lead to browning, such as the Maillard reaction, or cause the degradation of pigments like chlorophyll. Since chlorophyll is thermally unstable at high temperatures (Wen et al., 2025), among other factors, the process used to obtain the edible leathers can influence the final product's characteristic color, potentially resulting in chlorophyll degradation and a loss of the green color.

Table 1
Luminosity (L[a]), chroma a[a], chroma b[a], chroma (C[a]), browning index (BI), and visual evaluation of edible leathers produced with different concentrations of ora-pro-nóbis leaf puree (C_{OLP}).

C_{OLP}	Visual appearance	L[a]	Chroma a[a]	Chroma b[a]	C[a]	BI
30		39.11 ± 1.83[a]	1.93 ± 0.10[a]	10.73 ± 0.71[a]	10.91 ± 0.70[a]	35.22 ± 3.15[a]
40		38.68 ± 1.40[a]	1.85 ± 0.09[a]	9.97 ± 0.42[b]	10.14 ± 0.42[b]	32.85 ± 2.13[a]
50		37.30 ± 2.18[a]	1.91 ± 0.17[a]	10.22 ± 0.92[ab]	10.40 ± 0.93[ab]	35.25 ± 3.24[a]
60		38.18 ± 1.80[a]	1.84 ± 0.12[a]	10.34 ± 0.70[ab]	10.50 ± 0.69[ab]	34.62 ± 2.82[a]

[a] Different lowercase letters in the same column indicate a significant difference ($p < 0.05$).

Table 2
Water activity (Aw), moisture content, protein content, hygroscopicity, and pH of edible leathers produced with different ora-pro-nobis leaf puree concentrations (C_{OLP}).

C_{OLP}	Aw	Moistue[a]	Potein[a]	Hygoscopicity[b]	pH
30	0.748 ± 0.02[ab]	7.49 ± 0.61[a]	6.80 ± 0.12[cd]	28.07 ± 1.02[a]	4.80 ± 0.08[ab]
40	0.752 ± 0.02[ab]	7.77 ± 0.56[a]	8.08 ± 0.08[b]	24.27 ± 0.46[a]	4.75 ± 0.03[b]
50	0.762 ± 0.06[a]	7.33 ± 0.22[a]	8.68 ± 0.07[a]	27.27 ± 2.02[a]	4.82 ± 0.03[a]
60	0.742 ± 0.08[b]	6.52 ± 0.63[a]	8.95 ± 0.14[a]	26.84 ± 0.55[a]	4.75 ± 0.01[ab]

* Different lowercase letters in the same column indicate a significant difference ($p < 0.05$).
[a] (g/100g w.b.).
[b] (g of water absorbed/100 g of dry matter).

3.2. Water activity (Aw), humidity, proteins, hygroscopicity, and pH

The water activity of the edible leathers, regardless of the concentration of ora-pro-nóbis, showed no significant difference ($p > 0.05$) (Table 2). Within this range, all products demonstrate a certain level of stability, as they are less susceptible to microbiological contamination. According to Maltini et al. (2003) [15], water activity is related to the majority of chemical, enzymatic, and physical degradation reactions in food products.

Humidity is an important parameter that directly influences the stability of food products. Among the developed formulations, the leathers presented reduced humidity (Table 2). These results suggest that edible leathers have the potential for a longer shelf life, especially when stored under appropriate temperature and humidity conditions, favoring the preservation of the texture properties of the final product.

Increasing OLP concentration resulted in a higher protein amount in the edible leathers, with the highest protein content observed in the formulation containing 80 C_{OLP} (Table 2). This was expected, as many reports in the literature indicate that ora-pro-nóbis leaves are a source of protein ([7,16]; Gomes et al., 2025). According to the FDA (2025), a food product can be classified as a good source of protein if it contains at least 5–95 g of protein per serving (10–20 % DV). Lysine constitutes 5.4 % of the protein content in ora-pro-nóbis leaves (Silva et al., 2017). Edible leathers with 50 and 60 g C_{OLP} presented ~8 % protein. Additionally, ora-pro-nóbis leaves are considered "neutral in taste" because they don't have a very characteristic flavor, which can contribute to their consumption.

[4] produced edible leathers (pestil) enriched with wheat, corn, and potato flours. They reported that, based on their experimental design, the highest protein content achieved (6.58 g/100 g) was observed in the formulation containing 12 g/100 g of wheat flour—a value lower than that found in the present study.

The hygroscopicity values (Table 2) showed no significant differences, indicating that increasing the concentration of ora-pro-nobis puree did not influence the water-absorption capacity of the edible leathers.

However, the pH presented a significant difference between the formulations ($p < 0.05$), possibly due to the compounds present in the leaves, which resulted in more acidic edible leathers. According to Takeiti et al. (2009) [17], ora-pro-nobis leaves are a source of vitamin C, which may have contributed to the lower pH in the edible leathers, considering that agar-agar has a neutral pH (6.5–7.5, according to the manufacturer).

Since it is a clean-label product (composed only of OLP and agar-agar), its composition and the concentration of active compounds may be influenced by several factors. Therefore, changes in the extraction method of bioactive compounds [18], the use of sweeteners [19], and modifications in the product's physical appearance [20] can directly impact its quality and functionality.

3.3. Thickness and perforation

The thickness of the edible leathers increased significantly with higher puree concentration, resulting in greater thickness after drying (Table 3). The puree also contains a high amount of mucilage [21]. According to Conceição et al. [22], the mucilage of ora-pro-nóbis leaves exhibits typical bands and peaks characteristic of polysaccharides with protein components, as indicated by infrared spectra. This presence may promote water retention during drying, significantly influencing the thickness of the edible leathers.

The texture is an important characteristic for characterizing leathers, especially regarding product acceptance. Typically, the penetration force increased as the ora-pro-nobis puree concentration increased. The highest penetration force was observed in the leathers produced with $C_{OLP=}$60 g, which had the greatest thickness (0.49 mm). It is possible that higher puree concentrations, despite structural differences, result in a more cohesive structure.

3.4. Scanning electron microscopy

All the formulations exhibited a heterogeneous surface, and the internal structure indicated the presence of discontinuity zones (Fig. 1). This structure may be associated with the presence of various compounds of OLP, such as proteins, dietary fibers, and mucilage [21]. These compounds, along with agar-agar, could alter the structural organization of the edible leathers, creating less cohesive areas and contributing to microcracks and collapsed zones throughout the material. According to Novianto et al. (2025) [23], the pure agar-agar films have a homogeneous surface structure, with no aggregation or breaks in the matrix, suggesting that the observed changes may result from the addition of OLP. Particles are also present within the internal structure, possibly related to unsolubilized water agar.

3.5. Nuclear magnetic resonance

Fig. 2 presents the results of nuclear magnetic resonance and cell viability of leathers with different concentrations of ora-pro-nóbis puree (5 (30 C_{OLP}), 6 (40 C_{OLP}), 7 (50 C_{OLP}) and 8 (60 C_{OLP})). Cross-polarization excitation of $_1H–_{13}C$ results in the positions of the lines are characteristic of each type of carbon in the molecule (Fig. 2a). The black spectrum corresponds to the agar-agar sample, while the red spectrum represents the ora-pro-nóbis puree sample. The most significant components present in ora-pro-nóbis were identified, including primarily hemicellulose (peaks in the δ = 25–175 ppm range), lignin (peaks in the δ = 60–125 ppm range), cellulose (peaks at around δ = 100 ppm, referring to cellulose C1), and possibly amino acids (peaks in the δ = 50–90 ppm region).

Compared to near-infrared (NIR) techniques, solid-state NMR may be less efficient and have lower sample throughput, but it provides more precise measurements and is less affected by interferences such as particle size variations, dyes, and surface structure. A solid-state NMR method was developed to quantify cellulose and polyester in textile blends, the technique proved accurate and applicable to various textile waste, including man-made cellulose fibers using sequence of pulse CP-MAS [24]. In the present work, there was an increase in the peak at δ = 50–90 ppm region ppm as the concentration of puree in the leathers increased (Fig. 2b), possibly referring exclusively to the CH_2 groups present in the amino acids from the ora-pro-nóbis. This indicates that the increase in the concentration of puree, as expected, causes an increase in the concentration of proteins.

Table 3
Thickness and perforation (Force and Distance) of edible leathers produced with different ora-pro-nóbis leaf puree concentrations (C_{OLP}).

C_{OLP}	Thickness (mm)	Force (N)	Distance (mm)
30	0.32 ± 0.02^{b}	3.27 ± 0.63^{c}	2.24 ± 1.09^{a}
40	0.38 ± 0.05^{a}	4.00 ± 0.68^{bc}	2.58 ± 0.58^{a}
50	0.37 ± 0.08^{a}	5.31 ± 0.14^{b}	2.21 ± 0.65^{a}
60	0.49 ± 0.07^{a}	7.45 ± 1.56^{a}	2.85 ± 1.12^{a}

* Different lowercase letters in the same column indicate a significant difference ($p < 0.05$).

3.6. Total phenolic compounds and ABTS•$^{+}$

The concentration of phenolic compounds increased significantly increasing C_{OLP} (Table 4). The same behavior was observed for the ABTS•$^{+}$ results, it was to be expected, since a higher concentration of puree provided a higher concentration of active compounds in edible leathers.

The ora-pro-nóbis leaves have a high antioxidant capacity and a high content of phenolic compounds [25]. According to Ferreira et al. [16], the main metabolites found in the leaves are carotenoids and phenolic compounds, including phenolic acids and flavonoids. Antioxidant compounds act as agents that reduce or eliminate free radicals, capable of neutralizing oxidative stress caused by excessive free radicals in the body.

[25] identified 10 phenolic compounds in the leaves of ora-pro-nóbis after hydroethanolic extraction. The main ones are citric acid, which constitutes more than 49 % of the phenolic content, followed by quercetin-3-O-rutinoside and isorhamnetin-O-pentoside-O-rutinoside [26]. reported 24 phenolic compounds, with the main ones being caffeic acid, ellagic acid, p-anisic acid, p-coumaric acid, kaempferol, and quercetin.

3.7. Cytotoxicity

The results obtained in the assessment of cell viability in HaCat cells treated with different concentrations of ora-pro-nobis puree (Fig. 3) indicate that edible leathers did not have a cytotoxic effect at the tested concentrations, as cell viability remained above 70 % in all formulations. According to ISO 10993-5 (2009), cell viability below 70 % indicates a potential cytotoxic effect. Therefore, edible leathers demonstrate a promising safety profile.

The leaves of ora-pro-nóbis are commonly used in both traditional Brazilian cuisine as a food and as a topical remedy in popular medicine; there are no reports in the literature about adverse effects from their consumption. Several studies in the literature report the non-toxicity of extracts from ora-pro-nóbis leaves, demonstrating a non-cytotoxic behavior in different cell lines, including both tumor and normal cells [13,27,28].

4. Conclusion

The production of edible leathers based on ora-pro-nóbis leaf puree represents an innovative and sustainable approach to the utilization of unconventional food plants (UFP). The results demonstrated that incorporating ora-pro-nóbis significantly enhances the nutritional value

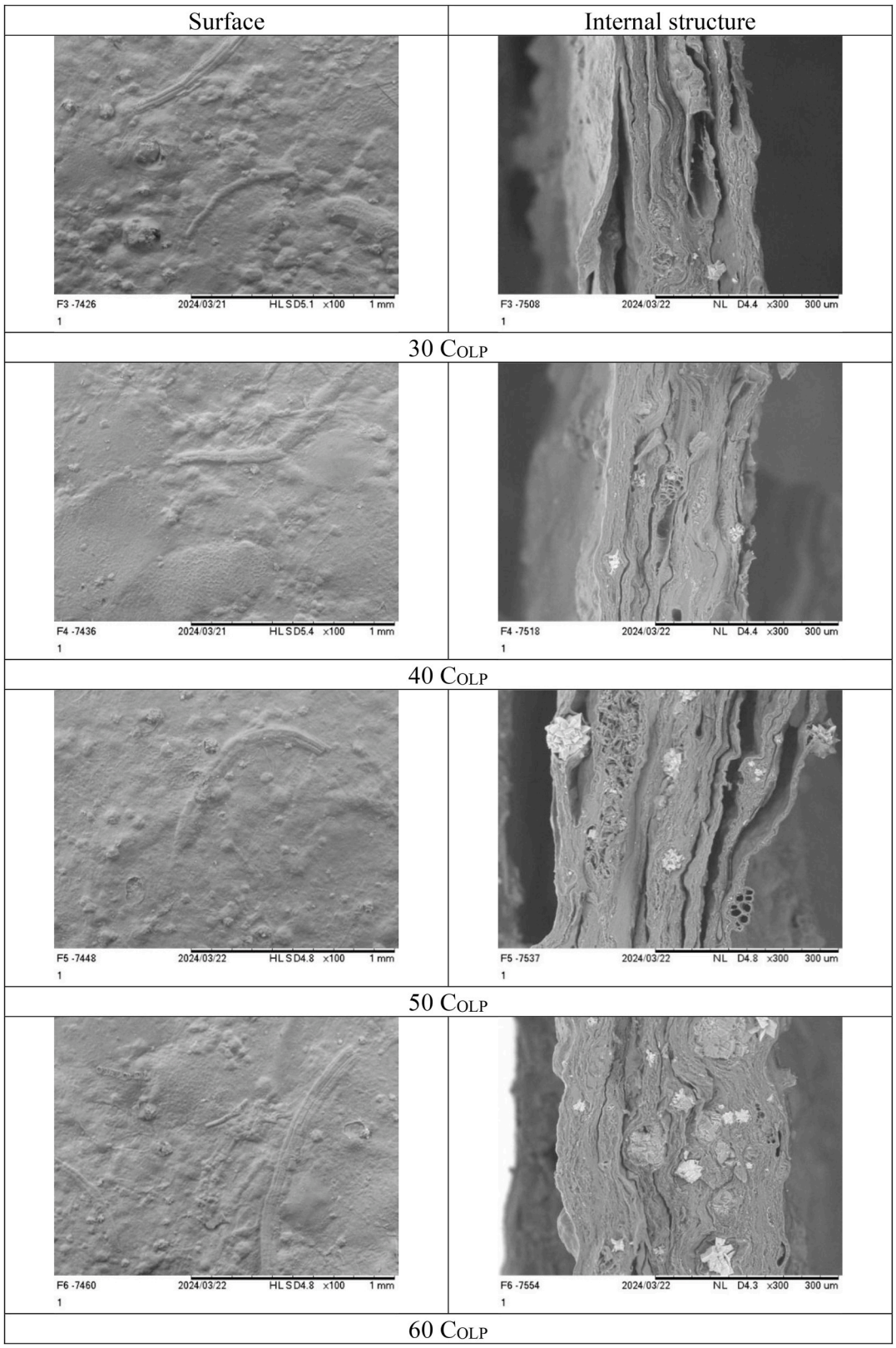

Fig. 1. Scanning electron microscopy (surface and cross-section) images of edible leathers produced with different concentrations of ora-pro-nóbis leaf puree (C_{OLP}).

of the final product, particularly by increasing protein content and antioxidant capacity, in addition to improving mechanical resistance and physicochemical stability. It is important to highlight that previous studies have reported the absence of toxicity in these products, reinforcing their safety for consumption. With these characteristics, edible leathers emerge as viable and promising alternatives to meet the growing consumer demand for healthier, functional, and sustainable food options.

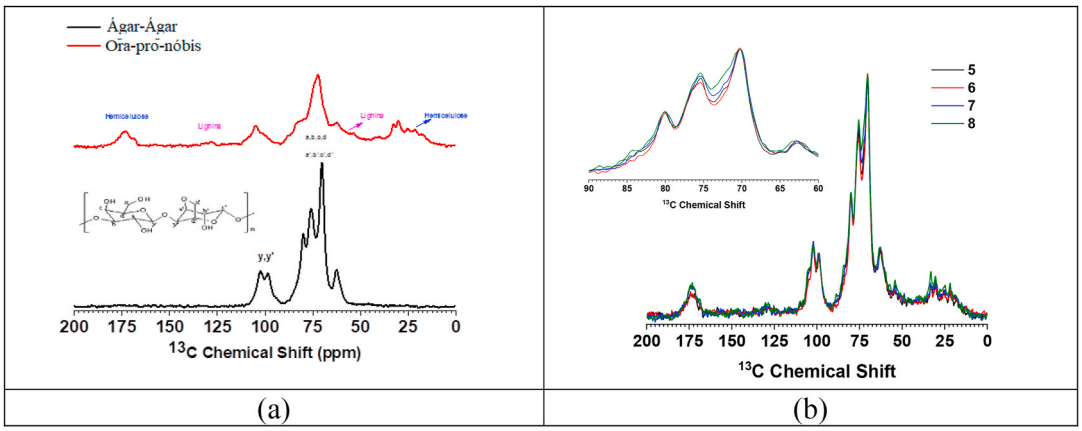

Fig. 2. Nuclear magnetic resonance (NMR) of edible leathers produced with different concentrations of ora-pro-nóbis leaf puree (C_{OLP}).

Table 4
Total phenolic compounds (TFC) e ABTS•$^{+}$ of edible leathers produced with different concentrations of ora-pro-nóbis leaf puree (C_{OLP}).

C_{OLP}	TFC (mg GAE/g edible leathers)	ABTS•$^{+}$ (μmol Trolox eq./100 g edible leathers)
30	680.35 ± 65.14^{c}	1272.15 ± 111.27^{a}
40	696.37 ± 61.41^{c}	1354.73 ± 75.86^{b}
50	770.81 ± 65.39^{b}	1440.99 ± 53.04^{c}
60	830.57 ± 91.71^{a}	1499.01 ± 74.28^{c}

*Different lowercase letters in the same column indicate a significant difference ($p < 0.05$).

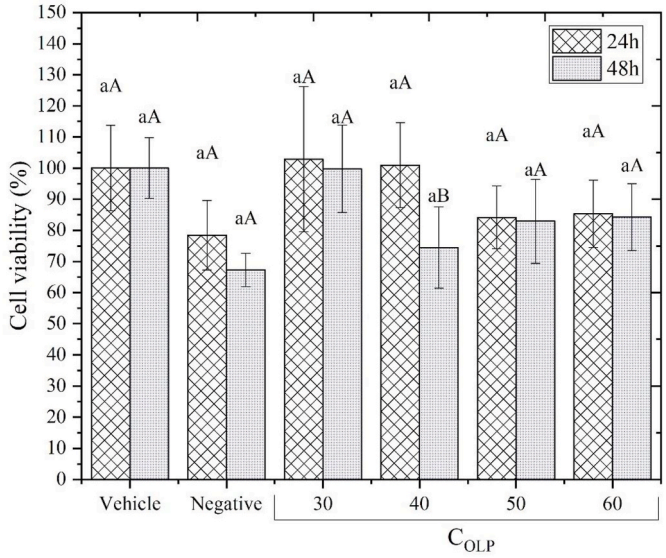

Fig. 3. Edible leathers (edible films) cytotoxicity produced with different concentrations of ora-pro-nóbis leaf puree (C_{OLP}) in 24 and 48 h.

CRediT authorship contribution statement

Tiago de Freitas Pereira: Writing – original draft, Methodology, Investigation, Formal analysis. **Rosemary Aparecida de Carvalho:** Writing – review & editing, Writing – original draft, Visualization. **Cristiana Maria Pedroso Yoshida:** Writing – review & editing, Writing – original draft, Data curation, Conceptualization. **Rodrigo Henrique dos Santos Garcia:** Writing – original draft, Methodology, Formal analysis. **Pricila Veiga-Santos:** Writing – original draft, Visualization, Validation. **Maria Luisa Gonçalves Agneis:** Methodology, Investigation, Formal analysis. **Fábio Rodrigues Ferreira Seiva:** Methodology, Investigation, Formal analysis. **Silvia Maria Martelli:** Writing – original draft, Visualization. **Danielle Marques Vilela:** Writing – original draft, Visualization. **Vitor Augusto dos Santos Garcia:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Abbreviations

OLP, ora-pro-nóbis leaf puree; ELFM, edible leather forming mass; SEM, Scanning Electron Microscopy; NMR, resonance magnetic nuclear; Aw, water activity; TFC, total phenolic compounds; C_{OLP}, concentrations of ora-pro-nóbis leaf puree; UFP, unconventional food plants; SDGs, Sustainable Development Goals.

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